Performance of QuantiFERON®-TB Gold In-Tube assay in children receiving disease modifying anti-rheumatic drugs

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Original article

Background: **To evaluate the performance of the Quantiferon®-TB Gold In-Tube (QFT-IT) interferon (IFN)-γ assay for the detection of latent tuberculosis infection (LTBI) in children receiving anti-rheumatic treatment in a tertiary referral hospital of Northern Greece.**

Methods: **A total of 79 consecutive children receiving anti-rheumatic treatment [of which 18 screened prior to anti-tumor necrosis factor (TNF)-α treatment] were tested using Mantoux tuberculin skin test (TST) and QFT-IT. Association of both tests with risk factors for latent tuberculosis and Bacillus Calmette-Guerin immunization was determined. Influence of age, TNF-α inhibitors, systemic corticosteroids, conventional disease modifying anti-rheumatic drugs (DMARDs) and total duration of therapy on the QFT-IT mitogen-induced response was evaluated.**

Results: **Agreement between TST and QFT-IT results was moderate (*k*=0.38). Frequency of QFT-IT indeterminate results was low (2.5%). In patients with risk factors for LTBI, the odds of a positive IFN-γ assay was increased by a factor of 27.6 (*P*=0.002), whereas there was no positive TST. There was a significant difference in the mitogen-induced IFN-γ secretion among various treatments (*P*=0.038). TNF-α inhibitors were associated with increased mitogen-induced IFN-γ secretion compared to monotherapy with conventional DMARDs (*P*=0.008). All children screened prior to anti-TNF-α treatment exhibited a negative QFT-IT and no active TB disease was detected during a 2-year follow-up.**

Conclusions: **QFT-IT may be a more reliable test than TST for detection of LTBI in children with rheumatic diseases receiving anti-rheumatic treatment. Drug regimen might influence the mitogen-induced IFN-γ secretion and the effect of TNF-α inhibitors might vary according to the specific agent administered.**

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interferon-γ release assay;
latent tuberculosis;
rheumatic disease;
tuberculin

Introduction

Immune-mediated inflammatory disorders (IMID) are a risk factor for reactivation of tuberculosis, particularly if associated with immunosuppressive therapy.[1-4] The incidence of tuberculosis reactivation is increased in patients treated with tumor necrosis factor-α (TNF-α) inhibitors.[5-8] Tuberculin skin test (TST) measures the magnitude of the delayed-type hypersensivity reaction to intradermal inoculation of purified protein derivative (PPD), and is commonly used as a measure of exposure to *Mycobacterium tuberculosis* (MTB). It suffers from low specificity, giving positive reaction in individuals previously immunized with Bacille Calmette-Guerin vaccine (BCG) or infected with nontuberculous mycobacteria,[9,10] and exhibits poor sensitivity in patients with immune mediated disorders,[8,11,12] especially those receiving immunosuppressive therapy.[13-15]

Interferon (INF)-γ release assays (IGRAs) detect either IFN-γ secreted by effector T cells in whole blood *ex vivo* (enzyme-linked immuno sorbent assay, ELISA) or the frequency of pre-sensitized MTB-specific T cells releasing IFN-γ isolated from blood mononuclear cells (enzyme-linked immunospot assay). IFN-γ is released in response to MTB-specific antigens, located in a specific genomic area (RD1), absent from BCG and most environmental mycobacteria.[16,17] IGRAs have higher specificity than TST and are associated with TB

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risk factors in immunocompetent adults.[9] Although recent studies support the IGRA use in young children in the diagnosis of latent tuberculosis infection (LTBI) in low TB-incidence settings,[18,19] current international guidelines do not recommend IGRAs in <5 year-old or immunocompromised children as there is evidence that young age and immunosuppression are associated with increased frequency of indeterminate results.[20-23]

At present, whether children with IMID need screening with IGRAs, TST or both is controversial.[1] In addition, the impact of various disease modifying anti-rheumatic drugs (DMARDs) as well as systemic corticosteroids on IGRAs performance still needs to be established.[1,8,10]

The aim of this prospective study was to compare MTB-specific IFN-γ ELISA and TST for the detection of LTBI in a cohort of children and adolescents with rheumatic diseases receiving anti-rheumatic agents including TNF-α inhibitors. Patients were recruited from outpatient clinic of a tertiary university hospital in Northern Greece, a country with low incidence of TB and where most of non-tuberculous mycobacterial species isolated are not cross-reactive with QuantiFERON®-TB Gold In-Tube (QFT-IT) specific TB antigens not carrying the RD1 region.[24] The influence of age, duration of treatment with DMARDs and systemic corticosteroids on the QFT-IT IFN-γ response to control mitogen was also evaluated.

Methods

Patients

Inclusion criteria

Children and adolescents treated with DMARDs or systemic corticosteroids whose parents gave informed consent were prospectively recruited from the Pediatric Rheumatology Outpatient Clinic from October 2011 to October 2012. The study was approved by the Ethics Committee of the Hospital. For each child, demographic characteristics, BCG immunization status was assessed according to the immunizations record and risk factors for LTBI were collected by interviewing the parents using a standardized case report form. Data on anti-rheumatic treatment regimens were obtained from patient medical records. The following conditions were considered as LTBI risk factors: birth or residence for >6 months in a country with high TB prevalence (>20 cases per 100 000 population),[25] history of TB in a close relative or TST positivity in siblings. Anti-rheumatic treatment regimens were classified as: systemic corticosteroids, conventional DMARDs including methotrexate, leflunomide, azathioprine, cyclosporine, hydroxychloroquine and mycophenolate mofetil, and biologic DMARDs including adalimumab and etanercept. All medications were given at their indicated doses.

Exclusion criteria

The duration of treatment with systemic corticosteroids or DMARDs sufficient to cause immune impairment in children is not well established. For the purpose of this study administration of systemic corticosteroids for less than 1 month or of conventional DMARDs for less than 6 months or of biologic DMARDs for less than 3 months excluded for exclusion from the study.

INF-γ ELISA assay and TST

QFT-IT (Cellestis, Carnegie, Australia) and TST were performed on the same day of the interview. QFT-IT procedure was executed as per manufacturer's instructions. Briefly, 1 mL of blood was collected in each set of three QFT-IT tubes consisting of a Nil tube (negative control), a TB antigen tube (sample) and a Mitogen tube (positive control). The collected blood was gently mixed by inverting each tube several times to ensure that the entire inner surface of the tube was coated with blood to dissolve antigens on tube walls. Within 2 hours of blood collection, tubes were incubated at 37°C for 16-24 hours. At the end of the incubation period, sample tubes were centrifuged at 3000×g for 15 minutes and stored at -20°C until QFT-IT ELISA test was performed in duplicate wells. Test results were interpreted as indicated in the Cellestis QFT-IT package insert. The test was positive if Nil was ≤8.0 IU/mL and TB antigen was ≥0.35 IU/mL and ≥25% of Nil value. The test was negative if Nil was ≤8.0 IU/mL, the mitogen was ≥0.5 IU/mL, and the TB antigen was <0.35 IU/mL. The test was indeterminate if 1) Nil was >8.0 IU/mL or 2) Nil was ≤8.0 IU/mL, mitogen was <0.5 IU/mL, and TB antigen was <0.35 IU/mL.

TST was performed using 2 TU/0.1 mL tuberculin PPD RT 23 SSI (Statens Serum Institut, Copenhagen, Denmark) inoculated intradermally. According to current guidelines for immunosuppressed patients an induration read after 48-72 hours of >5 mm was considered positive.[26] Children with positive TST or QFT-IT were referred to the pediatric TB clinic for further assessment including a chest X-ray. LTBI was diagnosed if one or both tests (TST and QFT-IT) were positive providing there was no radiological evidence of active TB infection and in the absence of symptoms or clinical signs suggesting of active disease.

Statistical analysis

SPSS version 16.0 was used. Odds ratios (ORs)

and their 95% confidence intervals (CIs) for factors associated with positive test results were estimated by binomial logistic regression analysis. Concordance between QFT-IT and TST was performed using Cohen's kappa coefficient (k). The differences of mitogen-induced IFN-γ levels between various treatments groups were analyzed using the non-parametric Mann-Whitney U test. Kruskall-Wallis test was performed if more than two groups were considered.

Results

A total of 79 children (51 females, 64.5%) were enrolled. Patients' characteristics are summarized in Table 1. All were residents of the local area with a median (interquartile range, IQR) age of 12 (6) years. Twenty-four children (30.4%) were BCG vaccinated. The vast majority (n=74, 93.7%) were children with established juvenile idiopathic arthritis. Median (IQR) duration of anti-rheumatic treatment was 48 (36) months. Medication regimens were distributed as follows: conventional DMARDs monotherapy (44.3%), combination of conventional DMARDs with biologic DMARDs (39.9%) or with systemic corticosteroids (12.7%), biologic DMARDs monotherapy (2.5%) and corticosteroids monotherapy (1.3%; Fig. 1). The most common conventional DMARD was methotrexate (n=67), in combination with TNF-α inhibitors in 31 (46.2%), with corticosteroids in 5 (7.5%), with another conventional DMARD in 14 (20.9%) or alone in 17 (25.4%). Corticosteroid dose was very low (median: 5.5 mg/day). Among the biologic DMARDs (n=33), adalimumab was used in combination with methotrexate in 16 patients (48.5%) and alone in one (3%); etanercept was used in combination with methotrexate in 15 patients (45.5%) and alone in one (3%); no patient was receiving infliximab. Seven children (8.9%) had risk factors for LTBI: one was a contact of confirmed active TB, two had suspected TB family history and four were born in high TB incidence countries.

QFT-IT was positive in 3 patients (3.8%), negative in 74 (93.7%) and indeterminate in 2 (2.5%). Positive samples were retested to validate the result and considered positive only after confirmation. Of the 3 children who tested positive, two were considered at risk for LTBI (suspected TB family history). The two indeterminate QFT-IT results were both due to insufficient response to mitogen, none of the two children had risk factors for LTBI and both were on corticosteroids. TST was positive in 2 children (2.5%) and negative in 77 (97.5%). None of the two positive children had risk factors for LTBI, one of them also tested positive with QFT-IT. All children with positive QFT-IT or TST were given prophylaxis (2 with 9

Table 1. Characteristics of the study population

Patients' characteristics	Value
No. of patients	79
Female gender, *n* (%)	51 (64.5)
Age (y), median (IQR)	12 (6)
BCG immunized, *n* (%)	24 (30.4)
Risk factors for LTBI, *n* (%)	7 (8.9)
Diagnosis, *n* (%)	
Juvenile rheumatoid arthritis	74 (93.7)
Systemic lupus erythematosus	5 (6.3)
Treatment regimen, *n* (%)	
Conventional DMARDs only	35 (44.3)
Conventional and biologic DMARDs	31 (39.2)
Conventional DMARDs and steroids	10 (12.7)
Biologic DMARDs only	2 (2.5)
Steroids only	1 (1.3)
Treatment duration (y), median (IQR)	4 (3)

IQR: interquartile range; BCG: Bacille Calmette-Guerin vaccine;LTBI: latent tuberculosis infection; DMARDs: disease modifying anti-rheumatic drugs.

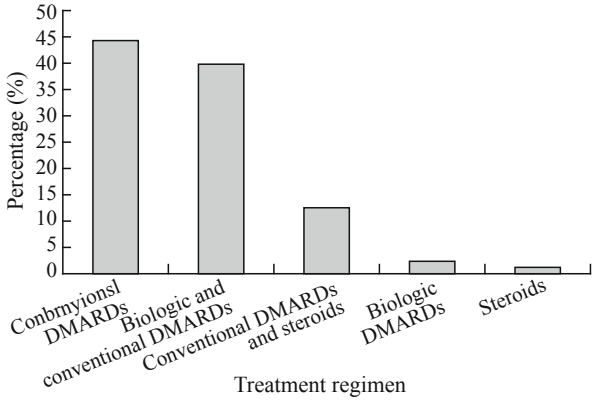

Fig. 1. Distribution of treatment regimen categories. DMARDs: disease modifying anti-rheumatic drugs.

Table 2. Association between positive results of QFT-IT or TST and presence of risk factors, BCG immunization status and duration of treatment during univariate analysis

Variables	Odds ratio (95% CI)	*P* value
QFT-IT positive		
Presence of risk factor*	27.6 (2.1-59)	0.021
Duration of treatment	0.82 (0.2-3.23)	0.770
BCG vaccination	1.18 (0.1-13.7)	0.890
TST positive		
Presence of risk factor*	NA	NA
Duration of treatment	0.82 (1.12-5.58)	0.840
BCG vaccination	2.41 (0.14-40.2)	0.890

*: including children born in or having parents from high incidence country,[25] TST positivity in siblings, or reported TB family history. QFT-IT: Quantiferon®-TB Gold In-Tube; TST: tuberculin skin test; BCG: Bacille Calmette-Guerin vaccine; TB: tuberculosis; CI: confidence interval; NA: not available.

months rifampicin including the double positive, 2 with 3 months isoniazid and rifampicin). The agreement between the two tests was moderate (k=0.38). In patients with risk factors for LTBI the odds of a positive IFN-γ assay from univariate logistic regression was increased by a factor of 27.6 (95% CI=2.9-59; P=0.021). No significant association was detected between BCG status or duration of treatment and a positive TST or QFT-IT result (Table 2).

Mitogen-induced IFN-γ level was not significantly associated with age (P=0.16). There was no significant association between a positive QFT-IT result and TNF-α inhibitors therapy (OR=2.77, 95% CI=0.24-32, P=0.57), and none of the two children with indeterminate QFT-IT results was receiving anti-TNF-α therapy. A significant difference was found in the mitogen-induced IFN-γ level among different treatment regimens (P=0.038). Treatment regimen with biologic DMARDs in combination with conventional DMARDs was significantly associated with increased mitogen-induced IFN-γ concentration as compared to treatment regimen with conventional DMARDs alone (P=0.0086; Fig. 2). Treatment with corticosteroids in combination with DMARDs was not associated with a decreased mitogen-induced IFN-γ level as compared to treatment with conventional DMARDs only (P=0.19).

Eighteen children (22.8%) were screened prior to the initiation of biologic DMARDs and also underwent a chest X-ray. All children tested negative with both screening tests, and did not show radiographic abnormalities. At two-year follow-up none of the 18 children showed any clinical or radiological signs of tuberculosis.

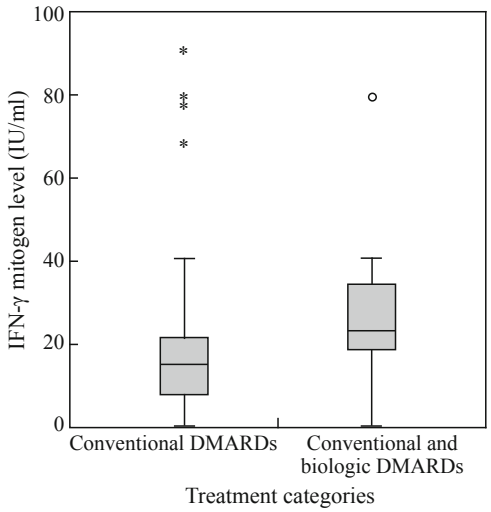

Fig. 2. Effect of conventional and biologic DMARDs on IFN-γ mitogen level. Box plot of the effect of biologic DMARDs on IFN-γ mitogen level; the bottom and top of the box are the lower and upper quartiles, the median is represented by a short black line within the box, the bottom and the top of the whisker represent the lower and upper extreme, whereas single data points (asterisks or circles) represent outliers. DMARDs: disease modifying anti-rheumatic drugs.

Discussion

To our knowledge, this is the first study evaluating the association of QFT-IT and TST with the presence of LTBI risk factors in Caucasian children receiving anti-rheumatic agents in a country with a TB incidence considered low according to the International Union Against Tuberculosis and Lung Disease Criteria.[25,27] Although few children were found to be at risk for LTBI, there was a significant association between positive QFT-IT results and presence of risk factors for LTBI; in addition, none of the children at risk of LTBI who tested positive with QFT-IT (n=2) would have been identified using TST screening only. This is in accordance with the findings of studies in adult patients with rheumatic diseases.[28-30]

The rate of indeterminate QFT-IT results (2.5%) is lower than those reported in studies evaluating the performance of IGRAs in immunocompromised patients.[15,21,22,31-33] All the above studies, except two[32,33] evaluated the performance of QFT-IT, and found a high rate of indeterminate results in this particular population. Children were the main target population only in one Italian study[22] and were included together with adult patients in a study from UK assessing the influence of age and immune status on the mitogen-induced IFN-γ level.[21] The performance of QFT-IT is assessed through the use of an internal positive control, which measures the IFN-γ response against phytohaemagglutinin (PHA) to exclude false negative results. The positive control is especially important when assessing patients on immunosuppressive medications prone to false negative results with both TST and IGRAs because of impaired immunity.[1,13,34] A low response against this mitogen in the context of negative response against the MTB-specific antigens leads to an indeterminate result. High rates of indeterminate results have been specifically associated with severe immunosuppression (e.g., chemotherapy for malignancy), treatment with high doses of corticosteroids or low CD4 counts.[22,33,35,36] The discrepancy of our finding may be due to the relatively low underlying immunosuppression in patients with pediatric rheumatic diseases and the contemporary judicious doses of conventional and biologic DMARDS that lead to immunomodulation and not immunosuppression in these populations. Previous studies that exclusively enrolled patients with IMIDs (adults or children) have reported similar rates of indeterminate results (less than 5%).[1,37] Only one recent study has reported a high rate (18%) of IGRAs indeterminate results

in the rheumatic adult population, although all patients with indeterminate responses were taking a higher median dose of corticosteroids.[38] The association between corticosteroids dosage and indeterminate results has been previously described.[35,39,40] In our study, the median dose of corticosteroids was very low (5.5 mg/day) and this may explain why no significant association between corticosteroid therapy and decrease of the mitogen induced IFN-γ production was found.

Our findings did not show any significant influence of age on the mitogen-induced IFN-γ level. However, we should notice that the small number of children of ≤5 years of age (n=7) did not allow accurate statistical judgments for this.

The finding of a significant increase of the mitogen-induced IFN-γ levels in children treated with anti-TNF-α therapy (either adalimumab or etanercept) is discordant to publications involving adult patients. In adults, treatment with infliximab, a monoclonal antibody not administered to our patients, has been previously associated with a decreased concentration of mitogen-induced IFN-γ in the positive control of IFN-γ ELISA-based assays and decreased odds for a positive result.[29,41] These reports align with the evidence that infliximab administered *in vivo* rapidly decreased the memory CD4+ T lymphocytes releasing IFN-γ upon challenge with mycobacterial antigens.[42,43]

We subsequently speculate that as our patients were treated with etanercept or adalimumab, in an equal rate, none of these agents had a greater impact in the mitogen-induced IFN-γ level. There is, however, evidence that TNF-α antagonists administered *in vitro* at therapeutic concentrations have differential effects on whole blood cell cultures stimulated with PHA or MTB-specific antigens when measured with IFN-γ ELISA-based assays.[44] While monoclonal anti-TNFα antibodies, such as infliximab and in a less extent adalimumab, are found to be associated with a significant decrease of antigen and mitogen T-cell activation, etanercept has no significant effect.[44] These findings could explain the reduced tuberculosis activation risk associated with etanercept as compared to infliximab.[44,45] Of note, etanercept and adalimumab administered to adult patients with rheumatoid arthritis have been reported to increase the IFN-γ production and the percentage of IFN-γ producing natural killer cells, respectively.[46,47] The hypothesis of a differential effect of TNF-α antagonists on IFN-γ production may provide a baseline explanation for our findings. However, the variety of study populations, materials, measurement methods and nature of the stimulus used limits the comparison of the results. To our knowledge, this is the first study to analyze the association between anti-TNF-α therapy and ELISA mitogen-induced IFN-γ response in children with rheumatic diseases.

There are two limitations in this study. The sample size of children, especially those under 5 years of age, is relatively small. However, although rheumatic diseases are relatively infrequent in young children, we have enrolled all eligible consecutive patients during the study protocol. The second limitation is the low percentage of children found to be at LTBI risk; however, as Greece is a low TB incidence setting,[27] this was an expected finding.

In conclusion, our findings suggest that QFT-IT may be more reliable than TST in identifying children with LTBI receiving anti-rheumatic treatment. TNF-α antagonists appear to have differential effects on the mitogen-induced IFN-γ level in the IFN-γ release ELISA-based assays. Prospective multicenter studies are required to determine the true predictive value of IGRAs and evaluate the potential impact of TNF-α antagonists and other DMARDs drugs on the performance of IGRAs in children receiving anti-rheumatic agents.

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Ethical approval: The study was approved by the Ethics Committee of the Hippokration General Hospital. Parents of study participants were given oral informed consent.

Competing interest: None declared.

Contributors: Gabriele F designed the protocol, conducted the study and wrote the initial draft. Trachana M, Simitsopoulou M and Pratsidou-Gertsi P conducted the study and reviewed the manuscript. Iosifidis E and Pana ZD analyzed data and reviewed the manuscript. Roilides E designed the protocol, conducted the study and reviewed the manuscript. All authors approved the final version of the manuscript.

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